

ESTCP

Cost and Performance Report

(ER-200513)



**A Low-Cost, Passive Approach for
Bacterial Growth and Distribution
for Large-Scale Implementation of
Bioaugmentation**

July 2012



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TECHNOLOGY CERTIFICATION PROGRAM

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ACRONYMS AND ABBREVIATIONS

µg/L	micrograms per liter
bgs	below ground surface
CDM	CDM Smith
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
cis-DCE	<i>cis</i> -1,2-dichloroethene
CMT	Continuous Multichannel Tubing
COD	chemical oxygen demand
CSIA	carbon stable isotope analysis
CVOC	chlorinated volatile organic compounds
DCE	1,2-dichloroethene
DHC	<i>Dehalococcoides</i>
DNA	deoxyribonucleic acid
DNAPL	dense, non-aqueous phase liquid
DoD	Department of Defense
EAB	enhanced anaerobic biodegradation
ESTCP	Environmental Security Technology Certification Program
ft/d	feet per day
gpm	gallons per minute
IR	Installation Restoration
ITRC	Interstate Technology & Regulatory Council
L	liter
m	meters
MCL	maximum contaminant level
mg/L	milligrams per liter
mV	millivolt
NASA	National Aeronautics and Space Administration
NAVFAC ESC	Naval Facilities Engineering Command Engineering Service Center
NAVFAC SW	Naval Facilities Engineering Command Southwest
NAVWPNSTA	Naval Weapons Station
NPL	National Priorities List
O&M	operations and maintenance
ODC	other direct cost
ORP	oxidation/reduction potential

ACRONYMS AND ABBREVIATIONS (continued)

PCE	tetrachloroethene
qPCR	quantitative polymerase chain reaction
RCRA	Resource Conservation and Recovery Act
SDWA	Safe Drinking Water Act
TCE <i>trans</i> -DCE	trichloroethene <i>trans</i> -1,2-dichloroethene
USEPA	U.S. Environmental Protection Agency
VC	vinyl chloride
VOC	volatile organic compound
WDR	Waste Discharge Requirement

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1.0 EXECUTIVE SUMMARY

1.1 OBJECTIVES OF THE DEMONSTRATION

The overall objective of this work is to compare the cost and performance of full-scale bioaugmentation of chlorinated solvent contaminated groundwater using passive and active bacterial distribution approaches. The relative pros and cons of active recirculation and passive inject-and-drift strategies for large-scale bioaugmentation of chlorinated solvents in groundwater were evaluated in a side-by-side comparison at the Seal Beach Naval Weapons Station (NAVWPNSTA) Seal Beach Site 70 in the City of Seal Beach, CA. Three phases of activities were completed for each of the treatment cells, as follows:

- Phase 1 – Pre-demonstration Laboratory investigations
- Phase 2 – Tracer test, baseline sampling, and preconditioning
- Phase 3 – Bioaugmentation and monitoring.

1.2 DEMONSTRATION RESULTS

Bench-scale testing showed that complete dechlorination of trichloroethene (TCE) to ethene could be achieved even in the presence of high concentrations of sulfate, as long as sulfate-reducing conditions prevailed. While deoxyribonucleic acid (DNA) analysis revealed low concentrations of native *Dehalococcoides* (*DHC*) species at the site in a few locations, it was determined that not all of the known functional genes for dechlorination were present. Specifically, the *vcrA* gene was absent in site groundwater. As this functional gene is present in commercially available dechlorination cultures, it was tentatively selected as an appropriate biomarker for the bioaugmented culture pending results of DNA analysis of groundwater samples following the preconditioning phase.

Baseline groundwater sampling confirmed that initial conditions were mildly reducing, with high concentrations of TCE (maximum concentrations of 140,000 micrograms per liter [$\mu\text{g}/\text{L}$] for the active cell and 60,000 $\mu\text{g}/\text{L}$ in the passive cell), with very little conversion to *cis*-1,2-dichloroethene (*cis*-DCE). During preconditioning, electron donor was distributed throughout most of the passive cell and throughout the upgradient portion of the active cell. Where electron donor was distributed, sulfate-reducing conditions were generally achieved, and in some locations, TCE transformation to *cis*-DCE was observed. However, almost no vinyl chloride (VC) was detected, and *DHC* detections were few and at very low concentrations. Most importantly for the DNA analysis of groundwater samples, no detections of the *vcrA* functional gene were observed, confirming its utility as a biomarker of the bioaugmentation culture.

Bioaugmentation of both treatment cells occurred in January 2009. Following bioaugmentation and during injection of 1% sodium lactate, considerable increases in numbers of *DHC* bacteria (ranging from $>10^6$ gene copies/liter [L] to $>10^9$ gene copies/L) and all three functional genes (*tceA*, *bvcA*, and *vcrA*) were observed in all wells in the upper portion of the active cell. Overall, conversion of TCE to ethene was proceeding effectively in the upgradient third to half of the active treatment cell but was not observed at the monitoring well two-thirds of the way down the treatment cell axis.

In the passive treatment cell, the electron donor distribution appeared to improve over time using the original monthly injection frequency. During the post-bioaugmentation phase, TCE and 1,2-dichloroethene (DCE) were mostly removed, with VC and ethene observed for the first time at injection wells PIW-2 and -3 within 2 weeks after inoculation in January 2009. As of October 2009, total chlorinated volatile organic compounds (CVOC) continue to remain low at all three injection wells. However, little to no dechlorination was observed in the upper portion of the passive cell during the post-bioaugmentation phase, possibly due to inhibition of dechlorination due to the presence of other contaminants such as chloroform. In contrast, complete reductive dechlorination of TCE to ethene was observed in the central and lower portion of the passive cell.

The growth of *DHC* was measured in each cell using DNA analysis of groundwater samples based on the total number of cells at the end of the study compared to the number injected, as well as by tracking increases over time at monitoring wells. Growth was very similar in both cells, with about a two order of magnitude increase in cell numbers estimated in each. It was also observed that concentrations at injection wells were sustained above about 10^6 gene copies/L throughout the test, and concentrations at monitoring wells increased to concentrations approximately equal to the injection wells by the end of the test. As with the first measure of growth, the two bioaugmentation strategies appeared equally effective based on this analysis.

Comparing and contrasting the distribution of *DHC* by the two bioaugmentation strategies was the key objective of this demonstration. Based on previous studies of bacterial transport in general, and bioaugmentation specifically, groundwater velocity appeared to be one of only a few parameters than can be easily manipulated during bioremediation that might have a significant impact on transport of *DHC*. Relative distribution efficiency of passive versus active transport was assessed by comparing travel time of injected *DHC* to travel time of the conservative tracer (iodide) used in Phase 2 of the demonstration. The groundwater velocity in the active cell was 1 to 1.8 feet per day (ft/d), and for the passive cell it was 0.22 to 0.44 ft/d, a difference of approximately a factor of 5. The tracer and *DHC* data indicated that bacterial transport was not significantly retarded compared to groundwater flow in either the active or passive cells. In fact, first arrival of *DHC* was faster than that of the conservative tracer in the majority of the passive cell monitoring wells. In the active cell, *DHC* transport velocity appeared to be approximately equal to that of the conservative tracer. These results demonstrate that *DHC* was transported more rapidly relative to groundwater flow under passive conditions than active recirculation. This is consistent with previous indications that retardation of *DHC* transport relative to a conservative tracer increases with groundwater velocity. The net result was that the passive distribution strategy provided effective distribution of *DHC* (along with complete dechlorination to ethene) over a larger portion of the treatment cell than was achieved with active recirculation.

1.3 COST ANALYSIS

Projected implementation costs for a “typical” application (not including the intensive monitoring required for a rigorous demonstration) of bioaugmentation at a 0.5-acre site using the active and passive approach were estimated based on the demonstration costs. Most of the costs are similar (e.g., start-up, general construction, monitoring, and performance assessment) because they are common to both active and passive approaches. However, the construction and

operations and maintenance (O&M) costs for the active approach are approximately three times as high as for the passive approach. The result is an estimated cost for the active approach of \$2.5 million, compared to \$1.5 million for the passive approach. The primary drivers for this cost increase are the significantly higher amount of lactate required, and the higher costs for construction and maintenance of recirculation systems. For a site like Seal Beach, the benefits of implementing an active recirculation approach do not appear to be justified by the increased costs.

It should be noted, however, that some sites have conditions that would lead to more significant benefits for recirculation systems. For sites with very high groundwater flow velocities, recirculation might be needed to manage residence time within the treatment zone to avoid potential offsite migration of partially chlorinated by-products such as *cis*-DCE and VC. Such a site would also allow electron donor to be distributed over a much larger distance prior to being degraded than was possible at Seal Beach, which would also increase the benefit. On the other hand, sites with very low groundwater velocities might make a passive system impractical because very little distribution can be achieved without enhancing the hydraulic gradient. What this demonstration indicates is that for sites that are closer to the average in terms of groundwater velocity, passive bioaugmentation systems are likely to be more cost-effective than active systems.

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2.0 INTRODUCTION

This report provides the cost and performance data for full-scale bioaugmentation systems designed to transform chlorinated ethenes to ethene in groundwater. In particular, this report demonstrates the relative pros and cons of active recirculation and passive inject-and-drift strategies as a side-by-side comparison between the two approaches for large-scale bioaugmentation of chlorinated solvents in groundwater at the Seal Beach NAVWPNSTA Site 70 in the City of Seal Beach, CA. This project is sponsored by the Environmental Security Technology Certification Program (ESTCP) Project ER-200513, with additional funds provided by Naval Facilities Engineering Command Southwest (NAVFAC SW). The principal investigator for this project is Mr. Joey Trotsky from Naval Facilities Engineering Command Engineering Services Center (NAVFAC ESC), and the co-principal investigator is Dr. Kent Sorenson of CDM Smith (CDM). CDM is a demonstration partner under contract number N68711-05-C-0063.

2.1 BACKGROUND

Chlorinated solvents are the most common class of contaminants in groundwater at hazardous waste sites in the United States. While significant progress has been made in addressing solvent sites, parties responsible for cleaning up sites with chlorinated solvents in groundwater are still faced with several technologies with significant capital costs, secondary waste streams, the involvement of hazardous materials, and the potential for additional worker or environmental exposure. A more ideal technology would involve lower capital costs, would not generate secondary waste streams, would be nonhazardous to workers and the environment, would destroy contaminants *in situ*, would be low maintenance, and would minimize disturbance of the site.

Bioremediation has been identified as one of the major technologies that may be able to address this problem at chlorinated solvent sites. However, bacteria capable of complete dechlorination of chloroethenes to ethene are not always present at these sites, which can cause dechlorination to “stall” at *cis*-DCE. When this occurs, one mitigation strategy is to perform bioaugmentation, which is the introduction of bacteria capable of complete dechlorination to ethene into the affected groundwater. This process has only been successfully demonstrated at the pilot scale, however, and many issues related to full-scale implementation with important cost implications still need to be addressed.

Previous bioaugmentation pilot studies were conducted on the scale of tens of feet and used active recirculation for distribution of the bioaugmentation culture. The current demonstration complements and builds on pilot testing already completed by NAVFAC SW at NAVWPNSTA Seal Beach, Site 40 that successfully used a low-cost, passive approach for implementation of bioaugmentation. The purpose of this demonstration is to compare the low-cost, passive method for implementation of bioaugmentation to the active recirculation method for full-scale application at a scale of hundreds of feet or more.

2.2 OBJECTIVE OF THE DEMONSTRATION

The overall objective of this work is to compare the cost and performance of full-scale bioaugmentation of chlorinated solvent contaminated groundwater using passive and active distribution approaches. The technical objectives for this demonstration are as follows:

- Extend bioaugmentation cost-effectively to full scale
 - Demonstrate cost-effective bacterial distribution at a scale of greater than 100 ft, rather than tens of feet as has previously been demonstrated
 - Demonstrate induction of complete dechlorination at the same scale
- Demonstrate that a low-cost, passive approach to bioaugmentation will achieve large-scale bacterial distribution and induction of complete dechlorination
- Compare and contrast effectiveness of passive and active approaches of bacterial distribution.

Specific performance objectives for each test scenario are provided in Section 4.

2.3 REGULATORY DRIVERS

The presence of chlorinated solvents including tetrachloroethene (PCE), TCE, *cis*-DCE, *trans*-1,2-dichloroethene (*trans*-DCE), and VC in groundwater is one of the most persistent environmental problems at National Priorities List (NPL) sites, as discussed in Section 2.1. The Safe Drinking Water Act (SDWA) maximum contaminant levels (MCL) for these compounds are very low, as shown in Table 1, which makes cleanup of these sites difficult given that solubility can be six orders of magnitude above the MCL.

Table 1. Regulatory limits for chlorinated compounds.

Compound	Regulatory Limit (MCL)¹ mg/L	Solubility @ 25°C mg/L
Tetrachloroethene	0.005	150 ²
Trichloroethene	0.005	1,100 ²
<i>cis</i> -1,2-dichloroethene	0.07	3,500 ³
<i>trans</i> -1,2-dichloroethene	0.1	6,300 ²
Vinyl chloride	0.002	2,763 ⁴

¹40 CFR 141.61

²Knox et al., 1993

³Howard, 1990

⁴Howard, 1989

mg/L – milligrams per liter

3.0 TECHNOLOGY

The first publications describing field-scale bioaugmentation using *DHC* bacteria to treat chlorinated ethenes appeared in about 2000, making this a relatively new technology for full-scale field applications. This section provides an overview of the underlying theory that is fundamental for technology application and a brief comparison of the advantages and limitations of bioaugmentation relative to other source remediation technologies. Additional detail on this topic can be found in the project Final Report (ESTCP, 2010).

In general, bioaugmentation for remediation of chlorinated solvents involves addition of electron donor (biostimulation) and a bacterial culture that contains *DHC*. Different techniques are available for bioaugmentation of groundwater, and the appropriate technique depends not only on the relevant application (e.g., plume containment versus source treatment) but also on the electron donor selected. Because all bioaugmentation methods require the addition of electron donor, it is important to consider the electron donor delivery method when selecting a bioaugmentation approach. Several electron donor emplacement methodologies have been used for biostimulation, including conventional and direct push wells, trenching, and soil fracturing (Interstate Technology & Regulatory Council [ITRC], 2005).

3.1 TECHNOLOGY DESCRIPTION

Two important topics pertinent to application of the technology are reviewed briefly here. First, a discussion of the basics of chlorinated ethene degradation is provided. Second, issues related to scale-up of bioaugmentation are presented, including factors that can affect bacterial transport in the subsurface.

3.1.1 Chlorinated Ethene Degradation

Complete biological reductive dechlorination of PCE and TCE to ethene was first documented only 2 decades ago (Freedman and Gossett, 1989), and the pathway was observed to proceed as follows: PCE → TCE → DCE → VC → ethene. Complete reductive dechlorination generally has two requirements. First, redox conditions must be sufficiently reducing that reductive dechlorination of DCE and VC to ethene is thermodynamically favorable. When electron donor is limited, conditions will often not be sufficiently reducing to achieve complete dechlorination, causing it to “stall” at DCE. This can be overcome simply through the addition of a compound that acts as an electron donor, often consisting of a fermentable carbon source (Sorenson, 2003).

The second requirement for complete reductive dechlorination is a biological community capable of carrying out the reaction. An increasing body of evidence suggests that complete biological reductive dechlorination of PCE and TCE to ethene requires the presence of a strain of the bacterium *DHC* (Cupples et al., 2003; He et al., 2003; Hendrickson et al., 2002). Many recently discovered organisms are capable of reducing PCE and TCE to DCE (Holliger et al., 1999; Drzyzga and Gottschalk, 2002), but only *DHC* have been found to be capable of complete dechlorination of PCE and TCE to ethene in a pure culture (Maymó-Gatell et al., 1997; Maymó-Gatell et al., 1999; Maymó-Gatell and Zinder, 2001). Together, these studies suggest that, while *DHC* are relatively common and widely distributed, their absence at a site might prevent complete dechlorination.

3.1.2 Bioaugmentation Scale-Up Issues

Bioaugmentation, the *in situ* addition of an exogenous bacterial culture containing *DHC* (in this case) to site groundwater, is gaining acceptance as a viable strategy for remediation of chlorinated solvents in groundwater, especially when these bacteria are not naturally present at a site and reductive dechlorination is found to stall at *cis*-DCE. Several studies have demonstrated that bioaugmentation using *DHC*-containing mixed cultures can overcome DCE stall and facilitate complete dechlorination at the field pilot scale (Ellis et al., 2000; Lendvay et al., 2003; Major et al., 2002).

During pilot-scale demonstrations, *D. ethenogenes* has been further distributed after inoculation through forced advection (recirculation) systems. While these systems have been effective at transporting bacteria approximately 10 feet in 5 weeks (Lendvay et al., 2003) or up to 30 feet in 3 months (Major et al., 2002), larger scale distribution has not been well documented. Furthermore, the use of such systems on a scale of hundreds of feet would either require many injection and extraction wells to achieve distribution on a similar time scale, or would require much higher extraction rates. Thus, the cost of scale-up could be very high. At active sites, cost increases go beyond merely the scale because recirculation pipes must be installed across roads, railroad tracks, or utilities, all of which can be problematic. A further complication is that obtaining regulatory approval to extract and reinject contaminated groundwater remains challenging at many sites. In some cases treating the extracted water is required, which eliminates many of the benefits of bioremediation.

While the fundamental issues affecting transport of *DHC* (or bacteria in general) are not well understood, results from a recent study at NAVWPNSTA Site 40 (see Section 3.2) suggest that a passive distribution system (low velocity) may be far more cost-effective for scale-up than an active recirculation system (high velocity). This study further investigated these factors by comparing both active and passive distribution at full scale at the same field site, using side-by-side treatment cells. Results from this demonstration provide field-scale data regarding technical performance and cost effectiveness of using both approaches.

3.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Significant advantages of bioaugmentation technology in general include low risk to human health and the environment during implementation, low secondary waste generation, minimal impacts during operations, and overall risk reduction. In addition, when applied in a source area, bioaugmentation offers the potential for complete source cleanup using one technology without a requirement for separate polishing technologies, which is a significant advantage from a cost standpoint. Source removal technologies generally do not remove all of the chlorinated solvent present and rely on polishing technologies, including *in situ* bioremediation and monitored natural attenuation to achieve cleanup standards. *In situ* bioremediation with bioaugmentation integrates source removal and polishing, thereby facilitating attainment of cleanup goals by reducing the need for further infrastructure, treatability studies, modification of site conditions, etc., that may be required to implement a polishing technology following source removal.

Challenges for bioremediation can include any of the site-specific characteristics that limit application of many remedial technologies, including complex lithology, low permeability

media, and high concentrations of competing electrons acceptors. In addition, this technology is probably not applicable for sites contaminated by large volumes of free-phase dense, non-aqueous phase liquid (DNAPL) (ITRC, 2005). Finally, the generation of methane is common at bioremediation/bioaugmentation sites, as is the temporary production of VC. Both can partition into the vadose zone above the water table, which can be a concern if the contamination is present in shallow groundwater underneath buildings or utility corridors.

In addition to the general advantages and limitations for bioaugmentation discussed above, each bioaugmentation approach being tested in this demonstration has its own advantages and limitations. For active recirculation bioaugmentation, the most significant advantage is that it provides the most control over amendment distribution because the gradients can be manipulated. Other advantages include:

- The ability to achieve rapid initial donor distribution, which can lead to more rapid onset of reducing conditions
- Larger distribution from an individual injection point during injection
- Ability to add large amounts of amendments over a relatively short time frame.

The most significant disadvantage for active recirculation is that it generally has the highest capital costs and O&M requirements of any approach. Continual system monitoring, either by automated instrumentation, or by onsite staff, is needed to ensure that upset conditions are not encountered and that all aboveground equipment is operating as designed. In addition, logistical constraints at active facilities may impact placement of aboveground infrastructure.

The primary advantage to passive bioaugmentation is that it is a flexible approach that allows for more or less frequent applications of electron donor in locations that are not necessarily fixed, while keeping the operational requirements (and costs) low. Other advantages include:

- Ability to distribute and maintain high concentrations of electron donor to a large radius of influence from individual injection points
- Ability to perform frequent (i.e., monthly to quarterly) amendment injections cost effectively (on smaller scales)
- Large areas can be treated effectively with multiple injection points
- Minimal O&M and capital requirements compared to active recirculation.

The main disadvantage for the passive approach is that the primary distribution mechanism is ambient groundwater flow; the success of this injection technique is highly dependent on subsurface conditions at the site. If ambient groundwater is too slow, then the area treated using this approach may be limited. In addition, the time and number of injections required before reducing conditions are achieved can be significantly longer compared to an active recirculation system. Also, individual injections can take multiple days, depending on subsurface conditions.

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4.0 PERFORMANCE OBJECTIVES

This demonstration complemented work completed under the ESTCP project Bioaugmentation for Chlorinated Solvent Remediation: Microbial Transport, Growth, Survival and Dechlorinating Activity (ER-200315). It also built upon pilot testing completed by NAVFAC SW at NAVWPNSTA Site 40 that successfully used a low-cost, passive approach for implementation of bioaugmentation. As described in Section 3, the technical objectives for this project are as follows:

- Extend bioaugmentation cost-effectively to full scale
 - Demonstrate cost-effective bacterial distribution at scales of hundreds, rather than tens, of feet
 - Demonstrate induction of complete dechlorination at the same scale
- Demonstrate that a low-cost, passive approach to bioaugmentation will achieve large-scale bacterial distribution and induction of complete dechlorination
- Compare and contrast the effectiveness of passive and active approaches of bacterial distribution.

The critical performance elements to measure were the results of the Phase 1 laboratory studies, the effects of the Phase 2 biostimulation/preconditioning, and the distribution of bacteria and extent of dechlorination in each of the treatment cells during Phase 3. Thus, the parameters to be monitored include *DHC* cell counts, chloroethenes and metabolites, electron donor and fermentation products, bioactivity and redox indicators, and cost. The performance criteria are identified specifically in Table 2.

Table 2. Technology demonstration performance objectives.

Project Phase	Performance Objective	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives				
Phase 1: Demonstrate that selected bioaugmentation culture can overcome high sulfate conditions and perform dechlorination to ethene; select a bioaugmentation culture that contains <i>DHC</i> that can be distinguished from indigenous <i>DHC</i> .	Demonstrate that at least one commercially available bioaugmentation culture can carry out complete dechlorination in the presence of high sulfate concentrations. Determine if <i>DHC</i> are present onsite; if so select a culture that contains a <i>DHC</i> strain or functional gene not present naturally at site.	Electron donor, sulfate, chloroethene, and dissolved gas concentrations in bench-scale study Quantitative polymerase chain reaction (qPCR) results; DNA sequencing results	Production of ethene at concentrations at least 2x detection in bench study using site groundwater samples, reduction of 95% TCE Identification of a biomarker that is present in bioaugmentation culture(s) but not in native strains of <i>DHC</i>	Successful – see Section 7.1.1 Successful – see Section 7.1.2
Phase 2: Determine baseline conditions and precondition treatment cells.	Demonstrate that the layout and residence time of each treatment cell are such that demonstration performance can be meaningfully evaluated in a sufficient time. Demonstrate that electron donor can be adequately distributed to remove sulfate from the system and create strongly reducing conditions in both treatment cells.	Tracer compound (iodide) concentrations over time, groundwater velocity and direction, residence time Electron donor, sulfate, ferrous iron, and methane data to verify that whey injections have created strongly reducing conditions	Construct treatment cells such that travel time from injection wells to monitoring wells is 6 months or less Sulfate reducing conditions achieved at monitoring wells nearest to injection locations	Successful – see Section 7.2.1 Partially successful – see Section 7.2.2
Phase 3: Determine full-scale effectiveness of bacterial distribution using passive and active circulation systems.	Determine bacterial growth and distribution throughout the treatment cells using both bioaugmentation scenarios. Determine extent of dechlorination in both treatment cells during the test period.	qPCR analysis, iodide tracer Chloroethene and dissolved gas concentrations; stable carbon isotope analysis	Collect data that allow for quantitative assessment of tracer and bacterial transport time, and growth of bacteria over time Achieve full dechlorination to ethene using both approaches – detection of ethene at greater than 2x detection limit at greater than or equal to 2/3 of the monitoring wells in a given treatment cell	Successful – see Section 7.3.1 Partially successful – see Section 7.3.2
Qualitative Performance Objectives				
	Determine ease of use for both active and passive approaches.	Feedback from field personnel; injection and operational logs	Quantify operational requirements for each approach	Successful – see Section 7.4

5.0 SITE DESCRIPTION

This site description includes a discussion of the site location and history, geology and hydrogeology, and contaminant distribution. This includes site background conditions at the outset of the demonstration project, not including baseline characterization activities.

5.1 SITE LOCATION

NAVWPNSTA Installation Restoration (IR) Site 70 was the former National Aeronautics and Space Administration (NASA) Research Testing and Evaluation Area, a rocket engine test facility located just south of Westminster Boulevard and east of Seal Beach Boulevard in Seal Beach, CA (Figure 1). Site 70 encompasses approximately 40 acres on the northwestern quadrant of the NAVWPNSTA Seal Beach.

5.2 GEOLOGY AND HYDROGEOLOGY

The relevant hydrostratigraphic unit at the site for this demonstration is referred to as the upper fines unit. This unit extends from ground surface to approximately 60 ft below ground surface (bgs) and comprises three zones: a shallow zone of surficial soils and recent clayey sediments; an intermediate zone of interbedded silts, clays, and sandy silts and clays including a semi-perched zone; and a lower zone of interbedded silts, clays, and fine- to coarse-grained, silty to clayey sands. Based on cone penetrometer boring logs, fine- to medium-grained sands are present from approximately 20 to 30 ft bgs in the source area. These sands are underlain by a clay unit to about 40 ft bgs.

The water table in the source area was historically present at 5 to 12 ft. Based on historical data, the estimated conductivity in the Upper Fines Unit is 10 ft/d (Bechtel Environmental, Inc., 1999). Sitewide historical hydraulic gradients in the Upper Fines Unit ranged from 0.0002 to 0.0011. The gradient between wells EW-70-01 and MW-70-27 (in the demonstration area) ranges from 0.0012 to 0.0026, with the flow direction being from EW-10-01 toward MW-70-27 (Bechtel Environmental, Inc., 2005).

5.3 CONTAMINANT DISTRIBUTION

The groundwater plume at Site 70 contains primarily TCE and other volatile organic compounds (VOC) such as PCE, DCE, VC, chloroform, and others (Bechtel Environmental, Inc., 2005). The plume is estimated to be approximately 2400 ft long by 2000 ft wide and approximately 195 ft deep. The VOC plume consists of a small, high concentration source zone and a large area consisting of lower concentration dissolved VOCs. The location for this demonstration is the shallow source zone, and the estimated extent of TCE in the source zone is shown in Figure 2. Within the source area, some limited dechlorination has occurred, but the majority of contamination is present as TCE.

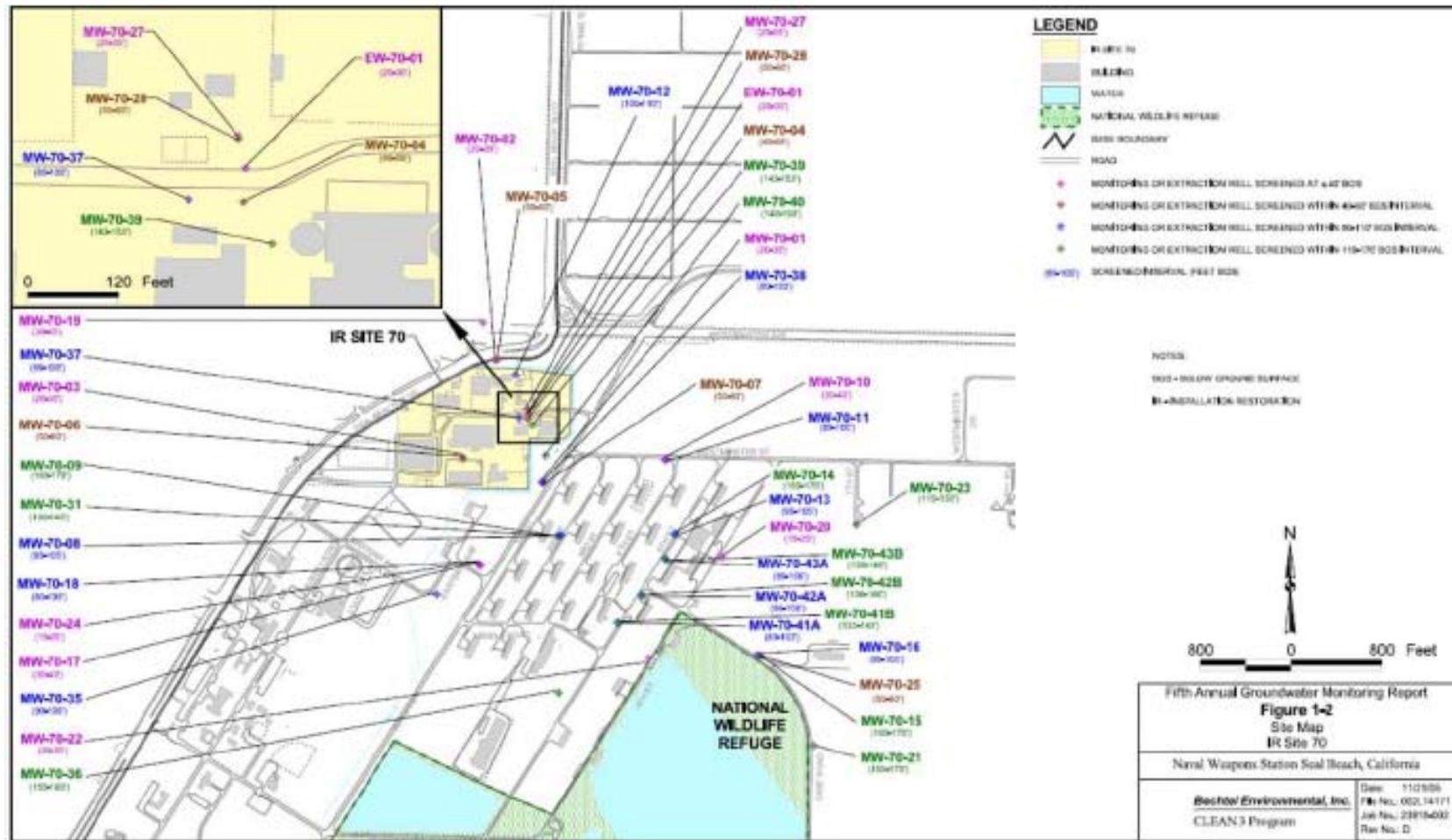


Figure 1. Site location map.

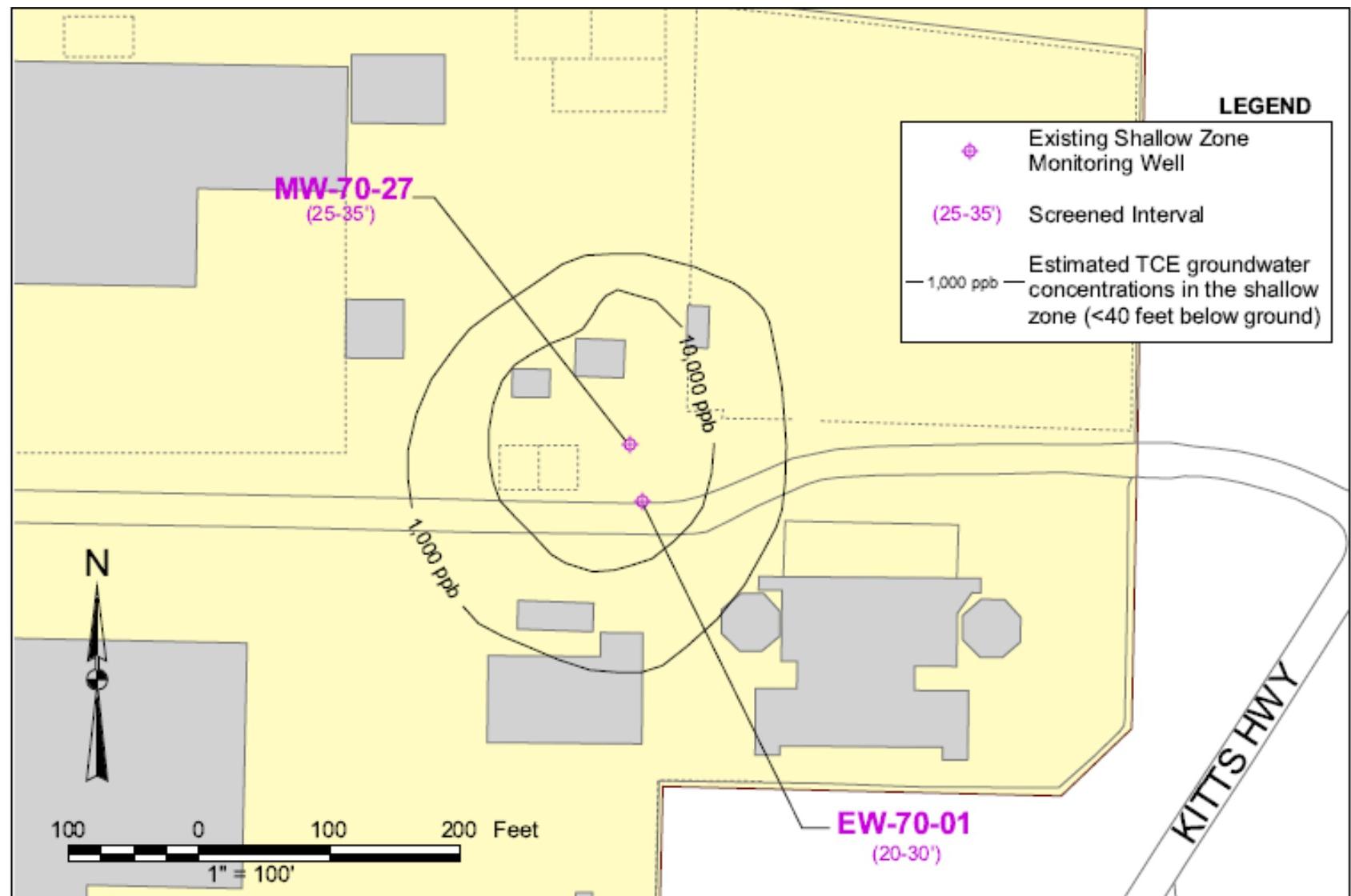


Figure 2. Estimated TCE source zone concentrations.

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6.0 TEST DESIGN

This section provides an overview of the system design and testing conducted during the demonstration. This includes the conceptual design, baseline characterization, treatability studies, field testing, sampling methods, and sampling results. For a more detailed description, see the project final technical report (ESTCP, 2010). Discussion and interpretation of the key results is provided in Section 7.

6.1 CONCEPTUAL EXPERIMENTAL DESIGN

The overall experimental design is based on the performance objectives presented in Section 4. The design comprised two independent treatment cells to test the passive and active bioaugmentation approaches in a side-by-side comparison. The passive treatment cell consists of three injection wells, three multilevel (Continuous Multichannel Tubing [CMT]) monitoring wells, and six standard monitoring wells. The active recirculation cell consists of two injection wells, two extraction/recirculation wells, three multilevel (CMT) monitoring wells, and three standard monitoring wells. The design was performed in three phases as described below:

Phase 1 – Pre-demonstration Laboratory Investigations. Bench-scale testing was performed to demonstrate that the bioaugmentation culture could overcome the high sulfate concentrations at the site. DNA analysis of site groundwater samples and commercially available cultures was used to identify “biomarkers” that provided the ability to differentiate between the injected cultures and any existing *DHC* that may have naturally existed in the groundwater.

Phase 2 - Tracer Test, Baseline Sampling, and Preconditioning. A tracer test and baseline sampling were used to assess baseline in both treatment cells. Electron donor was subsequently injected to create strongly reducing conditions and remove sulfate prior to bioaugmentation.

Phase 3 – Bioaugmentation and Monitoring. This third and final phase involved injecting the dechlorinating culture into each of the two treatment cells and performing groundwater monitoring to compare with results from Phase 2.

6.2 BASELINE CHARACTERIZATION

The objectives of the baseline characterization were to determine groundwater hydraulic conditions and baseline contaminant distribution, *DHC* distribution, and geochemical concentrations prior to beginning the biostimulation and bioaugmentation in each treatment cell. The treatment cell orientations were finalized as pertinent data for the shallow zone in this area were collected. The layout of both treatment cells as constructed is provided in Figure 3.

For the active treatment cell, concentrations were generally around 1000 to 3000 µg/L for TCE, with other contaminants present at low levels, but concentrations increased significantly at the southern end of the cell. The highest concentration measured anywhere in the ESTCP demonstration area was 140,000 µg/L at well AMW-6. The sample collected from the water being extracted from wells AEW-1 and AEW-2 had a TCE concentration of 10,000 µg/L.

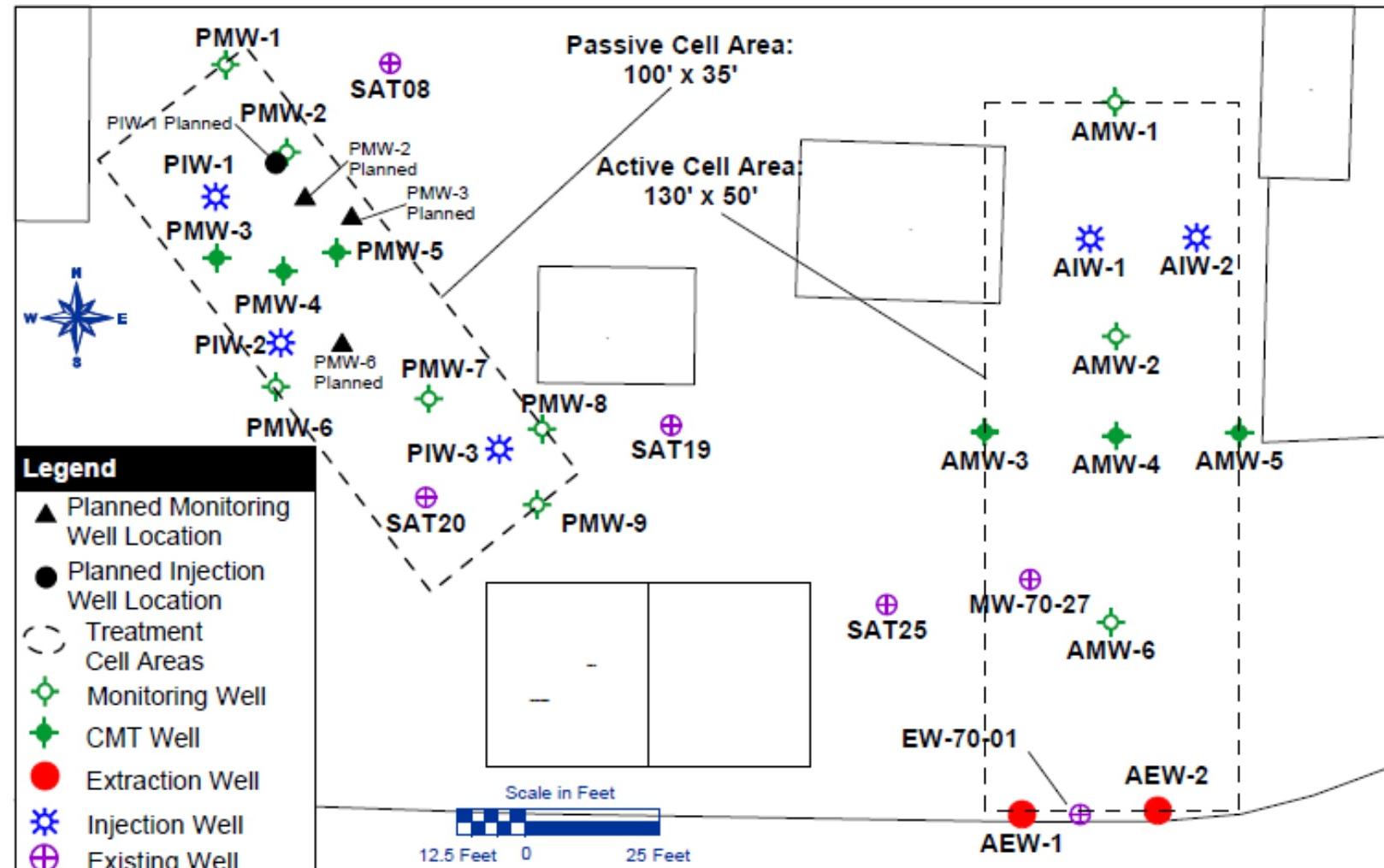


Figure 3. Well location map

For the passive cell, TCE concentrations were around 1000 µg/L at the ends of the treatment cell (wells PMW-1 and PMW-9); however, TCE concentrations were much higher in the center of the passive cell (15,000 µg/L to 63,000 µg/L). Concentrations of other VOC contaminants were low in all passive cell wells. Geochemical results (e.g., dissolved iron, sulfate, methane, etc.) in both cells showed mildly reducing conditions with low levels of available carbon. The pH was near neutral, and oxidation/reduction potential (ORP) ranged from 150 to +300 millivolts (mV).

6.3 TREATABILITY AND LABORATORY STUDY RESULTS

The Phase 1 laboratory studies successfully demonstrated that commercially available bioaugmentation cultures were able to perform complete dechlorination under high sulfate conditions. DNA analysis of field samples and commercial bioaugmentation cultures indicated that the functional reductase gene, *vcrA*, would be a good indicator of the bioaugmentation culture as it was not present in site groundwater samples (ESTCP, 2010).

6.4 FIELD TESTING

Field activities during this demonstration included system startup, preconditioning (Phase 2), bioaugmentation (Phase 3), and system shut down. Additional activities included temporary shutdown of the recirculation system and modification of lactate injections. This section includes a brief overview of all of these activities. See the final technical report for details (ESTCP, 2010).

6.4.1 System Start-Up

The recirculation system was ready for testing in March and April 2008. The system operated by extracting groundwater continually from wells AEW-1 and AEW-2 into a 275-gallon surge tank. The surge tank water was reinjected into AIW-1 and AIW-2, which were a distance of 100 ft upgradient from the extraction wells (refer to Figure 3 for well locations). The system was designed for groundwater flow rates in the range of 0.5 to 5 gallons per minute (gpm) per extraction well (1 to 10 gpm total). A proportional feed mixer was installed to pulse lactate into the recirculation line periodically as required. Instrumentation and controls were provided such that the system would run without an operator onsite.

6.4.2 Preconditioning

Preconditioning of the treatment cells was performed by injecting lactate to remove sulfate and create strongly reducing conditions prior to bioaugmentation. Lactate was injected every 4 weeks into the passive injection wells and quarterly into active injection wells. Preconditioning was completed successfully in January 2009.

6.4.3 Bioaugmentation

Following preconditioning, both the passive and active cells were inoculated with the SDC-9™ *DHC* culture in January 2009. Each cell was inoculated with approximately 100 L of SDC-9™. The inoculation was performed by injecting equal amounts of culture into each injection well (50 L per well in the active cell, 33 L per well in the passive cell).

Following the bioaugmentation, lactate injections were continued for 8 months. As carbon distribution was less than anticipated in the active cell, the pulsing strategy was modified from monthly to weekly during Phase 3. Although the frequency of injections was increased, the volume was decreased such that the monthly lactate mass injected did not change initially. In June 2009, the active cell lactate injection strategy was modified again to increase the lactate mass based on continued low carbon distribution throughout the active cell.

6.4.4 System Shut-Down

In October 2009, the recirculation system was shut down. Once it was determined in March 2010 that no additional data would be collected, the system was decommissioned in April 2010, and all equipment was removed from the site.

6.5 SAMPLING METHODS

Groundwater sampling was performed in each of the three phases of the demonstration to collect data to meet project objectives. Phase 1 included one round of sampling, and Phase 2 included three rounds. Following bioaugmentation, eight rounds of sampling were performed. All injection wells and monitoring wells (including CMT wells) were sampled in the passive cell during each event, and the combined effluent from the two extraction wells and all monitoring wells (including CMT wells) were sampled in the active cell during each event.

Analytical methods for this demonstration included standard U.S. Environmental Protection Agency (USEPA) methods for VOCs, ethene/ethane/methane, anions, chemical oxygen demand (COD), and alkalinity, as well as accepted field measurements using water quality instruments and field test kits. In addition, two innovative analytical techniques were used during this demonstration (qPCR and carbon stable isotope analysis [CSIA]).

6.6 SAMPLING RESULTS

Bioaugmentation performance, summarized in this section, was evaluated based on the extent of electron donor distribution, changes in redox conditions, extent and rate of dechlorination, and changes in the microbial population within the active and passive cells.

6.6.1 Active Cell

6.6.1.1 Electron Donor Distribution

Electron donor in the active cell was initially limited, resulting in multiple incremental changes in injection strategy that included increased injection frequency and higher concentrations and volumes. By the end of the demonstration, elevated COD concentrations in the range of a few hundred mg/L were observed at a number of monitoring wells. The donor distribution was greater than 36 ft downgradient of the injection wells and also included wells AMW-3 (Z2) and AMW-4 (Z2) but still failed to reach well AMW-6 located approximately 72 ft downgradient of the injection well. Continued effects of donor distribution were also observed approximately 25 ft upgradient of the injection well within the active cell.

6.6.1.2 Redox Conditions

Redox conditions in the active cell shifted in accordance with the electron donor distribution and, by the end of the demonstration, sulfate reducing to methanogenic conditions were established within the active cell except in the furthest downgradient locations, AMW-6 and the AEW wells.

6.6.1.3 VOC Concentrations

Following electron donor injections, an increase in TCE and total chloroethene concentrations was noted at all the wells sampled. This was likely caused by desorption and/or enhanced dissolution from residual nonaqueous TCE, and also due to the fact that TCE concentrations near the extraction well were higher than those near the injection wells at the start of recirculation.

Complete reductive dechlorination of TCE to ethene was observed in the upper half of the active cell following the increase in electron donor volume and concentration that began in June 2009. Figure 4 provides an example of dechlorination results; see ESTCP (2010) for all the data. As of October 2009, TCE degradation ranging from 85% to 99.7% was achieved in the upper portion of the active cell. In addition, large increases in VC concentrations and significant ethene production at wells AMW-1 and AMW-2, and all three zones of the three CMT wells, indicated that complete dechlorination was being achieved in the upper half of the active cell (greater than 36 ft downgradient and approximately 25 ft upgradient of the injection wells) as a function of electron donor distribution.

6.6.1.4 Biological Indicators

Dechlorinating bacteria, pH, and alkalinity can serve as indirect lines of evidence for occurrence of biological activity within the aquifer. In particular, an increase in numbers (i.e., growth) of dechlorinating bacteria suggests the occurrence of biodegradation of VOCs within the aquifer. These parameters are discussed below.

Dechlorinating Bacteria

During the preconditioning phase, low numbers of *DHC* bacteria (16S rRNA and/or functional genes *tceA* and *bvcA*) were observed at a few wells. However, the functional gene *vcrA* was not observed at any well within the active cell. Following bioaugmentation, considerable increases in numbers of *DHC* bacteria (ranging from >10⁶ gene copies/L to >10⁹ gene copies/L) and all three functional genes (*tceA*, *bvcA*, and *vcrA*) were observed in all wells in the upper portion of the active cell. Figure 5 provides an example of the *DHC* and functional gene data; see ESTCP (2010) for all the data. Low numbers of *DHC* bacteria observed at well AMW-6 and AEW indicated that these wells were not being impacted during the demonstration. While it cannot be stated that all the *DHC* present in the active cell were from the added culture, the *vcrA* results conclusively demonstrate that the bioaugmented *DHC* bacteria were transported to monitoring wells throughout the upper half of the active treatment cell (at least 36 ft downgradient).

Seal Beach
Groundwater Bioaugmentation

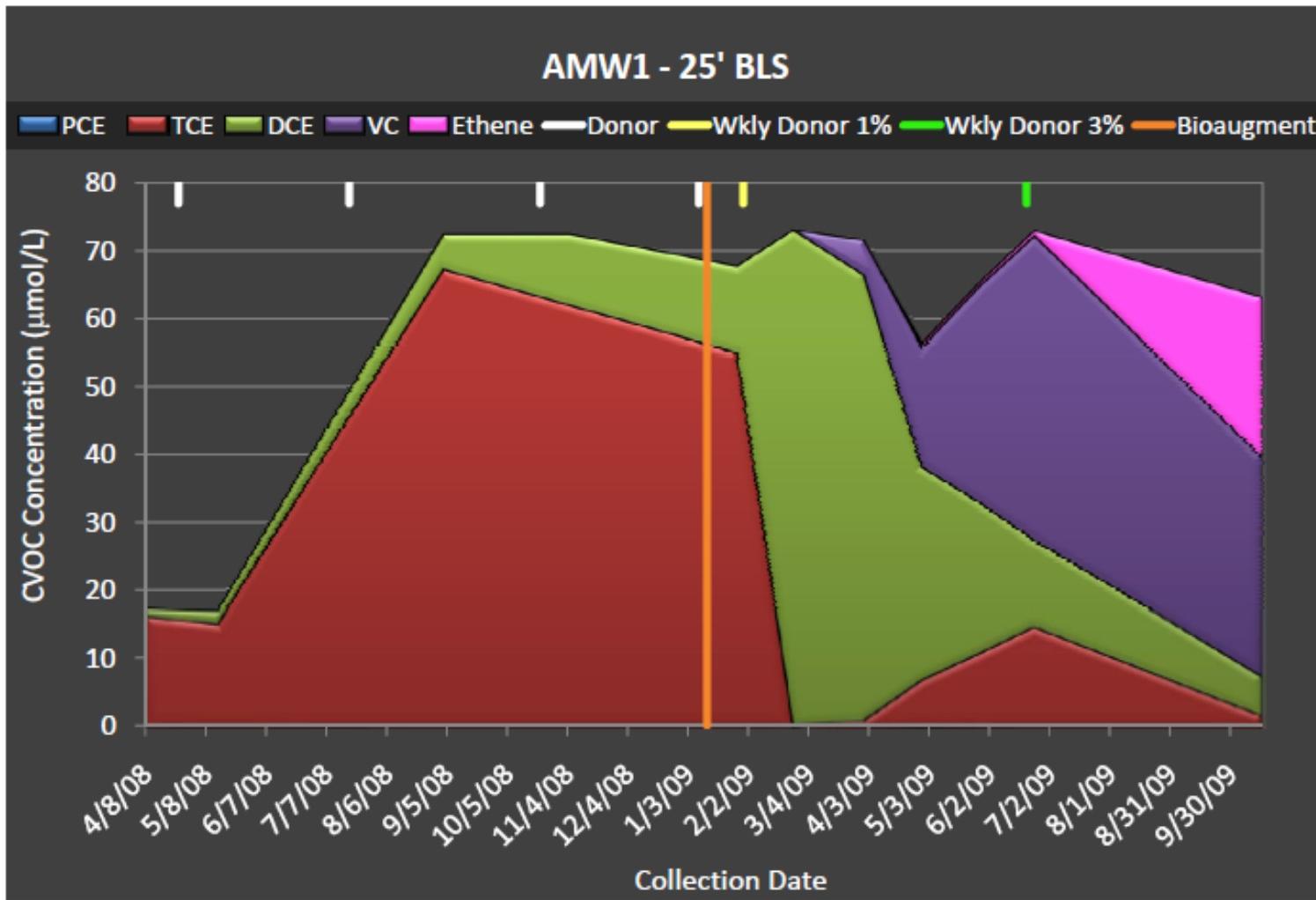
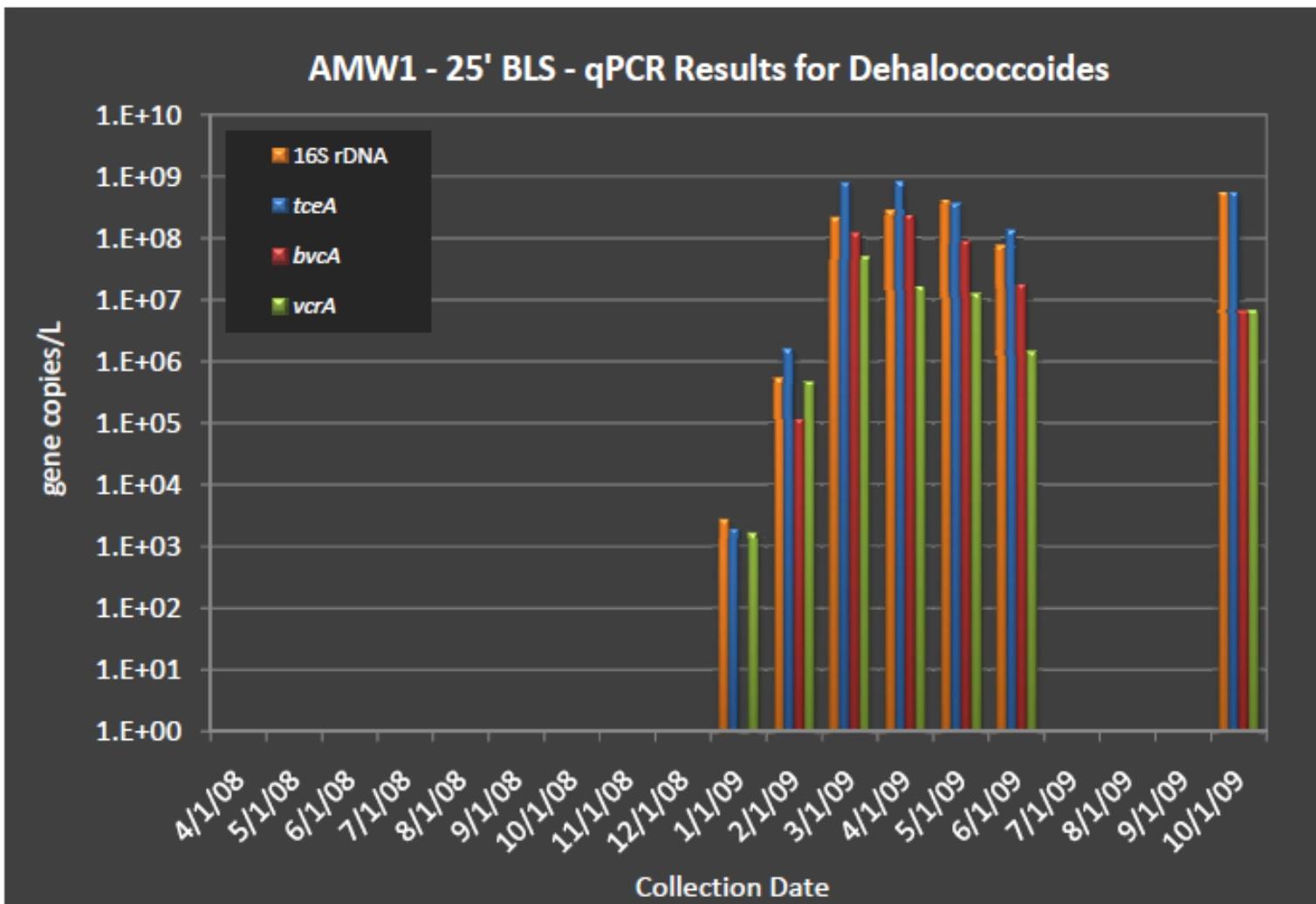


Figure 4. Chlorinated VOC molar concentration trend, AMW-1.

Seal Beach
Groundwater Bioaugmentation



Recirculation system was shut off between 9/2/2008 and 1/6/2009.

Figure 5. DHC concentration trend, AMW-1.

6.6.2 Passive Cell

6.6.2.1 Electron Donor Distribution

In general, COD concentrations increased to a few hundred to several thousands of mg/L in the passive cell, resulting in electron donor distribution approximately 22 ft downgradient and 15 feet cross-gradient of the individual injection wells. This facilitated distribution across almost the entire length of the passive cell with three injection wells. Effects of donor injections were observed a few months earlier in the central and lower portion of the passive cell compared to the upper portion. Vertical distribution appeared effective, with the impact of donor observed more in zones 2 and 3 compared to zone 1 of CMT wells.

6.6.2.2 Redox Conditions

Redox conditions in the passive cell shifted in accordance with the electron donor distribution, and as of October 2009, sulfate reducing to methanogenic conditions had been established within the passive cell. Unlike the active cell, no effects of donor injections were observed at the upgradient well, PMW-1, of the passive cell.

6.6.2.3 VOC Concentrations

During Phase 3, TCE and DCE were mostly removed, with VC and ethene observed for the first time at two of the injection wells within 2 weeks after inoculation in January 2009. Complete conversion of TCE to ethene was also observed at injection well PIW-1. As of October 2009, total CVOCs continued to remain low at all three injection wells.

Little to no dechlorination was observed in the upper portion of the passive cell during the post-bioaugmentation phase, despite the fact effective electron donor distribution and redox conditions appropriate for dechlorination were achieved. Chloroform detected only in this area of the demonstration might have inhibited dechlorination activity. In contrast, complete reductive dechlorination of TCE to ethene was observed in the central and lower portion of the passive cell. In October 2009 biodegradation accounted for reduction of total CVOC concentrations by greater than 92% at wells PMW-7 through PMW-9 and nearly 72% at well PMW-6. Ethene production was observed as high as 410 µg/L at wells PMW-6 through PMW-9.

6.6.2.4 Biological Indicators

Changes in numbers of dechlorinating bacteria and values of pH and alkalinity are discussed below.

Dechlorinating Bacteria

During the post-bioaugmentation phase, *DHC* bacteria and functional gene (*tceA* and *vcrA*) numbers increased immediately (within 2 weeks of inoculation) at all three injection wells to be on the order of >107 gene copies/L. As of October 2009, the numbers were observed to decrease by one to two orders of magnitude at the injection wells, suggesting that in the absence of high chloroethene concentrations, the *DHC* bacteria numbers might be decreasing. In the central and lower portion of the passive cell (wells PMW-6 through PMW-9) *DHC* bacteria and functional gene (*tceA* and *vcrA*) numbers increased to on the order of >108 gene copies/L and were

sustained as of October 2009. Only low detections of *DHC* bacteria and functional genes were observed in the most upgradient wells of the cell (PMW-1 and PMW-2). The vertical distribution in the CMT wells was variable, with Zone 1 generally having higher numbers than Zones 2 and 3.

Overall, the DNA results combined with the VOC data suggest that bioaugmentation was successful; i.e., dechlorinating bacteria were successfully distributed and maintained, and complete reductive dechlorination was achieved in the central and lower portions of the passive cell.

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7.0 PERFORMANCE ASSESSMENT

In this section, the implications of the results presented in Section 6 are discussed in the context of the project performance objectives.

7.1 PHASE 1 PERFORMANCE OBJECTIVES – BENCH SCALE TESTING AND BIOAUGMENTATION CULTURE SELECTION

The purpose of the Phase 1 of the ER-200513 project was to conduct laboratory studies to confirm that dechlorination could be stimulated in the high sulfate environment present at NAVWPNSTA Site 70, and to select a bioaugmentation culture for the demonstration. The sections below assess performance of the demonstration activities in achieving these objectives.

7.1.1 Demonstration of Dechlorination Using Site Groundwater

The results of the lab study showed that TCE was completely removed under all conditions investigated, which exceeded the goal of achieving at least 95% reduction of TCE. In addition, three of the four conditions tested met the criteria of production of ethene of at least twice the detection limit. Based on these results, the performance objective was met.

7.1.2 Select Bioaugmentation Culture with Reliable Biomarker

During the DNA study, several methods were used to characterize *DHC* populations, including quantitative PCR analysis and clone library analysis to evaluate various genes, including the 16S rRNA gene, and functional reductase genes *vcrA*, *bvcA*, and *tceA*. These analyses were performed for the 16S rRNA gene of NAVWPNSTA Site 70 indigenous *DHC* and three bioaugmentation cultures. The success criterion for this objective was identification of a biomarker that is present in bioaugmentation cultures but not in native *DHC*.

The results showed that the functional gene *vcrA* was not present at the site but was present in both the SDC-9™ and KB-1™ commercially available bioaugmentation cultures. Based on the fact that the SDC-9™ culture had been demonstrated to perform better in the presence of co-contaminants detected at Site 70 compared to KB-1™ (i.e., chloroform), the SDC-9™ culture was selected. Therefore, this performance objective was met.

7.2 PHASE 2 PERFORMANCE OBJECTIVES – BASELINE CONDITIONS AND PRECONDITIONING

The purpose of Phase 2 of the ER-200513 project was to determine groundwater hydraulic conditions and baseline contaminant distribution, *DHC* distribution, and geochemical concentrations prior to beginning the biostimulation and bioaugmentation in each treatment cell. Performance objectives were established related to demonstrating that the treatment cell layout was such that meaningful results could be obtained during the time frame of the project and to establishing appropriate conditions prior to bioaugmentation.

7.2.1 Treatment Cell Construction and Residence Time

The success criterion for this objective was to construct the treatment cells such that travel time from injection wells to monitoring wells was 6 months or less. In the active cell, arrival of tracer occurred within 6 weeks of injection for AMW-1 through AMW-5, including at the two deepest zones of all of the CMT wells. Tracer was not observed at well AMW-6 (75 ft from injection wells) during the time it was sampled.

For the passive cell, tracer arrival was observed within 4 weeks at the deepest interval in PMW-4 (center CMT well located 17 ft downgradient), at the deepest zones of PMW-3 and -5, and at cross-gradient well PMW-2 within 7 weeks. By the end of the passive cell tracer monitoring period of 3.5 months, tracer was measured at PMW-2 through PMW-5, including at the two deepest zones of all CMT wells.

Overall, the results of the tracer test showed arrival in some wells in less than one month in both treatment cells, and subsequent sampling for tracer indicated that travel times to all monitoring wells that were installed near the tracer injection wells were less than 4 months. This assured that meaningful data would be obtained within the 12-month planned duration of the demonstration. Therefore, this performance objective was met. In fact, as discussed in Section 7.3, the demonstration objectives were all met within a 9-month period.

7.2.2 Preconditioning Results

The success criterion for this objective was to create at least sulfate-reducing conditions at monitoring wells nearest to injection locations, such that the bioaugmentation culture would have a favorable environment following inoculation. After three lactate injections into the active cell, results indicated that appropriate conditions were achieved for successful bioaugmentation, particularly in wells near the reinjection locations. Ferrous iron increased to above 0.5 mg/L in all wells except AMW-6 and upgradient well AMW-1. Also, sulfate concentrations decreased more than 10% except in AMW-6 and the extraction wells. While COD concentrations did not increase above 60 mg/L in any active cell well, the significantly increased *cis*-DCE concentration at AMW-2 and other wells indicated that partial dechlorination was already occurring.

After three passive cell injections, results indicated that conditions were becoming more reducing, with the most positive results observed near the injection wells. At these wells, ferrous iron increased to above 0.5 mg/L and sulfate decreased more than 10% except in PMW-2 and PMW-6. COD increased significantly at wells near the injection points also, and significant COD still remained at two of the three injection wells.

Another key result from the sampling event immediately following preconditioning was that the *vcrA* functional gene was not detected at any location in either the active or passive cell, despite the fact that low concentrations of *DHC* did appear following the biostimulation phase. These results confirmed that the *vcrA* gene could be used to track the bioaugmentation culture.

Overall, the preconditioning results indicated that sufficient electron donor was being supplied for bioaugmentation and that redox conditions nearest the injection locations were sulfate reducing to methanogenic in both treatment cells. Therefore, this performance objective was met.

7.3 PHASE 3 PERFORMANCE OBJECTIVES – BIOAUGMENTATION RESULTS

The purpose of Phase 3 of the ER-200513 project was to demonstrate full-scale bioaugmentation and dechlorination using both the active and passive approaches. Phase 3 of the ER-200513 project began with inoculation of both treatment cells. Performance objectives were established related to collection of data that would allow for quantification of bacterial distribution and growth, and for assessment of the extent of dechlorination. These objectives are discussed further below.

7.3.1 Bacterial Growth and Distribution

The first Phase 3 objective was to assess and quantify bacterial growth and distribution in both treatment cells. Bacterial distribution was assessed by analyzing the first arrival of *DHC* bacteria (as measured by qPCR) at a given monitoring location following inoculation. This travel time was then compared to the travel time for ambient groundwater, as determined from the tracer test. Bacterial growth was assessed by analyzing the increase of *DHC* and functional gene counts at a given location once first arrival had been established. The success criterion for this objective was to collect data that allow for quantitative assessment of tracer and bacterial transport time and growth of bacteria over time. No specific criteria were set in terms of bacterial transport times or cell counts. Therefore, this performance objective was met. The subsections below quantify the arrival of tracer and bioaugmentation culture based on *vcrA* analysis.

In general, the distribution of *DHC* bacteria was effective in both the active and passive cells. *DHC* concentrations exceeded 10^8 gene copies/L in both treatment cells based on analysis of the 16S rRNA gene. In the active cell, the high *DHC* concentrations extended greater than 36 ft downgradient from the injections wells. In the passive cell, the high concentrations were distributed throughout the downgradient two-thirds of the cell. Perhaps more importantly, concentrations of the *vcrA* gene, indicated that the high *DHC* concentrations were representative of the bioaugmentation culture. The next two subsections discuss the speed at which the bacteria were distributed relative to groundwater velocity in the two cells.

7.3.1.1 Active Cell Distribution

The results from the active cell indicate that minimal retardation of *DHC* bacteria occurred compared to transport of conservative tracer. In terms of actual velocity, based on the distance from injection to monitoring wells, the average *DHC* velocity was 1.21 ft/d. Based on the tracer arrival, groundwater velocity using first arrival of tracer was 1.81 ft/d, while peak arrival yields a velocity of 0.97 ft/d. This implies that the *DHC* velocity was approximately the same as the actual groundwater velocity. Work published previous to this demonstration suggested that retardation factors of *DHC* under forced advection could be as high as 60-200 (Major et al., 2002). However, groundwater velocity for that study was much higher under the forced gradient (greater than 25 ft/d) than the current demonstration, which suggests that the increased retardation occurs only at high groundwater velocities (at least greater than 2 ft/d).

7.3.1.2 Passive Cell Distribution

For the passive cell, the retardation of *DHC* compared to peak tracer arrival was significantly less than 1, as the average was 0.13 with a standard deviation of 0.04. Even when compared to tracer first arrival, the retardation of *DHC* was 0.40 with a standard deviation of 0.16. This implies that the first arrival of bacteria was faster than the first arrival of tracer at all three CMT wells. Overall, the results indicate that bacterial transport in the passive cell was extremely rapid, with *DHC* arrival occurring faster than first arrival of conservative tracer at two of three monitoring locations. Perhaps the most important result is that bacterial transport in the passive cell was extremely rapid, with *DHC* colonization apparently occurring at distances of up to 30 ft from injection points within 2 to 5 weeks from inoculation.

7.3.1.3 Bacterial Transport Summary

The tracer and *DHC* data indicate that bacterial transport was not significantly retarded compared to groundwater flow in either the active or passive cells. The results further suggest that *DHC* were transported more rapidly relative to groundwater flow under passive conditions compared to active recirculation. The groundwater velocity in the active cell was 1 to 1.8 ft/d, and for the passive cell it was 0.22 to 0.44 ft/d. This is a contrast of approximately a factor of five, which represents a typical enhancement in flow that might be expected due to recirculation.

Another interesting observation was the fact that bacterial transport rate and extent was relatively independent of groundwater flow direction, especially in the passive cell. For example, cross-gradient wells such as PMW-2, PMW-7, and PMW-8 all showed *DHC* velocities similar to that of groundwater (0.2 to 0.3 ft/d). Therefore, *DHC* transport was not only less retarded in the direction of groundwater flow at slower groundwater velocities; it also occurred more rapidly in cross-gradient directions relative to the groundwater velocity (ESTCP, 2010).

Overall, the *DHC* results from both treatment cells are consistent with the analysis of previously published literature in ESTCP (2010) and support the hypothesis that *DHC* bacterial transport is affected by groundwater velocity (i.e., that retardation of bacteria decreases as groundwater velocity decreases.)

7.3.1.4 Bacterial Growth

Two methods were used to assess the extent of bacterial growth. The first was to quantify the number of *DHC* cells that were present at the end of the demonstration, and compare that to the number of cells added during bioaugmentation. During bioaugmentation, approximately 5×10^{12} total *DHC* cells were added to each treatment cell. By the end of the demonstration, both the active and passive treatment cells had approximately 10^{14} total *DHC* cells, which implies that growth of approximately two orders of magnitude was stimulated during the demonstration.

The second method was to determine whether *DHC* levels increased after first arrival at a given monitoring well. For both treatment cells, increases of two to five orders of magnitude of *DHC* concentrations following first arrival were observed at all locations that were monitored monthly.

7.3.2 Extent of Dechlorination

The second Phase 3 objective was to assess and quantify the extent of dechlorination using both the active and passive bioaugmentation approaches. The Final Report for the ER-200513 project (ESTCP, 2010) presents a full discussion of this objective in terms of decision rules that were established in the Demonstration Work Plan (CDM, 2007). A summary is included here.

In the active cell, complete dechlorination (as indicated by ethene production) occurred to a distance of at least 30 ft from the injection wells. By October 2009, VC and ethene were by far the predominant compounds at all locations within 30 ft of the injection wells. At 75 ft downgradient (AMW-6), degradation products were increasing at the end of the demonstration, but with no electron donor present and limited evidence of reducing conditions. This suggests that the presence of degradation products at this distance is simply due to migration from upgradient. Thus, complete dechlorination was stimulated to a distance between 30 and 75 ft. Based on these results, this objective was partially met for the active cell.

Complete dechlorination of TCE to ethene was achieved in the downgradient two-thirds of the passive treatment cell, with ethene remaining as the predominant product at several wells in October 2009. However, in the upper third of the cell, little dechlorination was observed in spite of having electron donor distributed to all the CMT wells, the fact that redox conditions were strongly reducing in this area, and low to moderate numbers of *DHC* were present. While determining the cause of this phenomenon was beyond the scope of this demonstration, it is very possible that inhibition from co-contaminants such as chloroform could have limited *DHC* activity. Chloroform was present at concentrations as high as 1500 µg/L and carbon tetrachloride as high as 15,000 µg/L in the passive cell near PIW-1. This is the only part of the demonstration area where these high concentrations were observed, and the only area where complete dechlorination was not achieved. Based on these results, this objective was partially met for the passive cell.

Overall then, this performance objective was partially met. What is more important, however, is that the data are more than sufficient to make a comparison of the relative pros and cons of the two bioaugmentation strategies.

7.3.3 Comparison of Performance of Active and Passive Approaches

Based on all data for both the active and passive treatment cells, the following conclusions can be made regarding technical performance of the demonstration:

- Electron donor distribution from an individual injection point was similar using both the passive and active approaches (greater than 25 ft in both cases).
- Electron donor and *DHC* distribution varied vertically for both strategies based on data from the CMT wells; this did not have a negative impact on dechlorination in the active cell, but dechlorination was minimal in all the CMT wells in the passive cell (likely due to inhibition caused by co-contaminants).
- Higher electron donor concentrations were achieved in the passive cell, which required significantly less donor compared to the active approach.

- Strongly reducing conditions were established within similar timeframes.
- Dechlorination performance was similar, with the exception of possible inhibition in part of the passive cell.
- Bacterial distribution was similar from a given injection location both in terms of time to first arrival and in terms of area influenced.

The passive approach stimulated dechlorination and bacterial distribution over a larger percentage of the treatment cell compared to the active approach, which was limited to the area near the injection wells. It is likely that the hydrogeology of this site played an important role in the similar technical performance of the passive and active bioaugmentation strategies. *DHC* first arrivals revealed the presence of some relatively high-flow solute transport pathways in the subsurface. It is possible that having some such higher-velocity flow paths is an important ingredient for the success of a passive bioaugmentation strategy. A tracer test is a useful characterization technique for any full-scale bioaugmentation application to assist not only in the selection of a passive versus an active approach but also for design of injection well spacing, placement of well screens, monitoring well locations, and so forth. Tracer testing with three-dimensional monitoring is particularly useful for this purpose, as was also documented in the final reports for ESTCP project ER-200218 (ESTCP, 2008).

Overall, technical performance of both approaches was similar in all regards. However, as discussed in Section 7.4, O&M requirements were much higher for the active approach. Also, as presented in Section 8, costs for the active approach were higher.

7.4 QUALITATIVE PERFORMANCE OBJECTIVES

The single qualitative performance objective established for the ER-200513 project was to assess the ease of use for both passive and active approaches. This includes operational time required in the field, time spent conducting maintenance and repair activities, and the amount of training required to operate each system. Data collected in support of this objective include feedback from field personnel, injection and operational logs, and the field team leader logbook.

During the course of the demonstration, the active recirculation system required much more time for troubleshooting and maintenance than the passive system. One major shutdown occurred in late 2008 due to malfunction of overflow shutoff switches and the autodialer (ESTCP, 2010). This required modification of the recirculation system to include an additional overflow tank and additional instrumentation. In addition, several minor equipment malfunctions occurred. Further, the active recirculation system required more training for field personnel regarding the Programmable Logic Controller program, how to dose the electron donor, and how to troubleshoot the system. Although it did not occur during this demonstration, it is our experience from working at other sites that biofouling is also more common in recirculation systems than passive injection systems.

In contrast, the passive system required no electronics, and had only one minor repair to replace flowmeters. Less training was required because the system consisted of a simple manifold to inject three wells at a time. The passive system did require a source of potable water for the injections, but this was available nearby.

The success criterion for this objective was to quantify the operational requirements for each approach. Data collected during the course of the ER-200513 demonstration did allow for an assessment of the ease of use of both approaches, and it was determined that the passive system was easier to use and required less maintenance. Therefore, this performance goal was met.

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8.0 COST ASSESSMENT

A critical evaluation criterion for any cleanup technology is cost. In this section, implementation costs for bioaugmentation of chlorinated solvent source areas are estimated based on the costs of the demonstration. Section 8.1 includes a review of the costs associated with the demonstration project. Section 8.2 provides a discussion of the primary cost drivers that influence effective implementation of enhanced anaerobic biodegradation (EAB), including a discussion of the positive and negative characteristics of active and passive treatment methods demonstrated during this project. Finally, Section 8.3 provides cost information for successful implementation of the remedy at a theoretical site.

8.1 COST MODEL

Table 3 provides the estimated implementation costs of the technology for the demonstration project at NAVWPNSTA Site 70. These are the approximate costs for performing a detailed demonstration of the technology, including more intensive sampling and analysis than would typically be needed for a more “standard” application of the technology. Projected costs for a more typical application of the technology at a model site are provided in Section 8.3. For clarity, a summary of each of the cost element tasks in Table 3 is provided following the table.

Table 3. Approximate implementation costs for EAB at NAVWPNSTA Site 70.

Cost Element	Sub-Category	Detail	Costs
Start-up costs	Treatability/DNA sequencing study	Procurement – 80 hr	\$6000
		Subcontractors (lab services)	\$20,000
	Work plan	Project manager – 220 hr	\$27,500
		Technical reviewer – 40 hr	\$8000
		Project engineer – 340 hr	\$34,000
		Drafting/clerical – 60 hr	\$4500
		Subtotal	\$100,000
General construction costs	Well installation/development	Project geologist – 500 hr	\$50,000
		Subcontractor	\$112,000
		Materials/other direct costs (ODC)	\$20,000
	Tracer testing/hydraulic characterization	Project manager – 40 hr	\$5000
		Project engineer – 40 hr	\$4000
		Project geologist – 160 hr	\$16,000
		Materials/ODCs	\$4200
	Screening level groundwater modeling	Project hydrogeologist – 24 hr	\$3000
		Subtotal	\$214,200
Active cell construction/ O&M	Oversight/supervision	Project manager – 200 hr	\$25,000
	Lactate injection system purchase/construction	Subcontractor	\$40,000
	Lactate injection (1x per week)	Project engineer – 10 hr/event, 40 events	\$40,000
		Lactate – 50 gal per event, 40 events	\$24,000
	Bioaugmentation	Project engineer – 20 hr	\$2000
		Bacterial culture	\$15,000
	System troubleshooting/ maintenance (1 major and 3 minor events during demo)	Project engineer – 80 hr	\$8000
		Technician – 80 hr	\$4800
		Materials/ODCs	\$10,000
	Sampling (12 total events)	Project engineer – 240 hr	\$24,000
		Project geologist – 240 hr	\$24,000
		Analytical (all analytes, including CSIA and qPCR)	\$106,500
		Materials/ODCs (\$1500 per event)	\$18,000
		Subtotal	\$341,300

Table 3. Approximate implementation costs for EAB at NAVWPNSTA Site 70 (continued).

Cost Element	Sub-Category	Detail	Costs
Passive cell construction/O&M	Oversight/supervision	Project manager – 100 hr	\$12,500
	Lactate injection system purchase/construction	Subcontractor	\$15,500
	Lactate injection (1x per week)	Project engineer – 20 hr/event, 12 events Lactate – 50 gal per event, 12 events	\$24,000 \$7200
	Bioaugmentation	Project engineer – 20 hr Bacterial culture	\$2000 \$15,000
	System troubleshooting/maintenance (1 minor event during demo)	Project engineer – 10 hr Technician – 10 hr	\$1000 \$600
	Sampling (12 total events)	Materials/ODCs Project engineer – 240 hr Project geologist – 240 hr Analytical (all analytes, including CSIA and qPCR)	\$1000 \$24,000 \$24,000 \$106,500
		Materials/ODCs (\$1,500 per event)	\$18,000
		Subtotal	\$251,300
	Includes final project reports, tech transfer, and data management/interpretation	Project manager – 600 hr Technical reviewer – 200 hr Project engineer – 600 hr Drafting/clerical – 200 hr Travel/ODCs	\$75,000 \$40,000 \$60,000 \$15,000 \$20,000
		Subtotal	\$210,000
Demobilization	Site cleanup and restoration		\$5000
Waste disposal			NA
Long-term monitoring			NA

ODC = other direct costs

Start-Up: consists of work plan development and treatability/DNA sequencing studies. Work plan development included finalization of the demonstration design and development of supporting documentation. The treatability study consisted of bench-scale testing for dechlorination recommended due to the high sulfate and chloride concentrations present at the site. The DNA sequencing study was conducted to determine whether native species of *DHC* were present at the site prior to the demonstration and to establish biomarkers for the injected culture.

General Construction: consists of well installation, tracer testing and hydraulic characterization, and groundwater modeling. Tracer testing and hydraulic characterization were performed to gather data on flow characteristics within both treatment cells. Modeling was performed to estimate groundwater extraction rates and to anticipate electron donor distribution.

Active Cell Construction: consists of injection system construction, lactate injections, bioaugmentation, system troubleshooting/maintenance, and sampling.

Passive Cell Construction: similar to the active cell construction, with the exception of groundwater extraction and reinjection. The passive cell did not include these components, and utilized natural groundwater flow to distribute electron donor and bacteria.

Performance Assessment, Reporting, and Project Management: includes ongoing management and review of analytical data, as well as periodic project reporting. This also includes preparation of the final project reports.

Demobilization: includes removing equipment and materials from the site as well as site restoration.

Waste Disposal: Includes removal and disposal of all investigation derived waste. These costs are standard, fairly insignificant, and were not tracked during the demonstration.

Long-Term Monitoring: Includes monitoring conducted after the demonstration is completed. These costs are standard and were not tracked during the demonstration.

8.2 COST DRIVERS

As with most in situ remediation technologies, the most important aspect of implementing bioaugmentation in chlorinated solvent source areas is delivery and distribution, that is, the electron donor and bacteria must be distributed throughout the target treatment zone to stimulate the desired degradation. Therefore, the major cost drivers are likely to be the infrastructure and materials required to achieve distribution of amendments. These are largely driven by the scale of a site laterally and vertically, as well as the hydraulic conductivity and the degree of heterogeneity. The “bulk” hydraulic conductivity of the treatment zone will determine the spacing of injection wells, and will have a strong influence on the required treatment duration. The heterogeneity will mostly impact the treatment duration because a high degree of heterogeneity will increase the potential for preferential flow. A high degree of preferential flow will result in a cleanup time frame that is dependent upon diffusion more than advection, which will increase treatment duration, thereby increasing costs.

Similarly, the sheer mass of contamination can be a cost driver. As long as the source consists primarily of solvents at residual saturation or sorbed to the soil, mass removal can be fairly rapid (subject to the potential constraints of hydraulic conductivity and heterogeneity discussed above). However, if DNAPL is present in pools, the cleanup time frame becomes limited by dissolution rates. While these rates can be accelerated during bioremediation (ESTCP, 2008), cleanup time frames will still be long for large pools of DNAPL.

Another potential cost driver is a need for hydraulic containment. If a sufficient downgradient buffer zone is not available at a site and extraction of groundwater is required to prevent the temporary increase in mass flux caused by EAB from impacting some nearby downgradient receptor, costs would increase. This is especially true if for some reason the extracted water cannot simply be reinjected in the source area.

Vapor intrusion concerns can also be a potential cost driver. Bioremediation of chlorinated solvents via EAB generates VC and methane. For shallow, unconfined groundwater sites, this creates the potential for these gases to reach fairly high concentrations in the unsaturated zone above the water table. If potential receptors were present above the treatment zone and soil vapor extraction were required, this would increase technology costs.

8.3 COST ANALYSIS

This section provides an estimate for typical passive and active bioaugmentation approaches at an example site with similar characteristics to that of NAVWPNSTA Site 70. This cost analysis focuses on comparing and contrasting these approaches for bioaugmentation in the context of implementing bioremediation for cleanup of a chlorinated solvent source area. For a comparison of bioremediation to other remediation technologies for source area cleanup, see the Cost and Performance Report for ESTCP Project ER-200218. This estimate is based on the costs associated with the demonstration project but does not include the level of rigor required for technology validation. Table 4 provides the site characteristics and assumptions for the example site.

Table 4. Parameters used as the basis for calculating technology implementation costs.

	Active Approach	Passive Approach
Site area (acre)	0.5	0.5
Site area (sq ft)	21,780	21,780
Contaminated thickness treated (ft)	20	20
Treatment volume (cubic yards)	16,200	16,200
Number of injection wells (scaled up from demonstration)	10	19
Number of multilevel monitoring wells	2	2
Number of fully penetrating monitoring wells	8	8
Number of extraction wells (active cell only)	10	0
Duration of operations (years)	5	5
Frequency/concentration of electron donor injection	weekly/3%	monthly/1%
Frequency of monitoring events	quarterly	quarterly
Monitoring analytes	Same as demonstration, but no CSIA and DNA except for first year	Same as demonstration, but no CSIA and DNA except for first year

An effort was made to be conservative in several of the parameters so as to avoid being too optimistic in the estimate. For example, the number of monitoring wells (especially the multilevel wells) is higher than many cleanups at the assumed scale. In addition, the Site 70 costs included tracer testing, modeling, a treatability study, and DNA sequencing, as noted in Table 3. These activities are not always performed in typical applications but can significantly improve technology performance and should be considered prior to implementation of a remedy.

In other cases, the demonstration costs were reduced to reflect, for example, the frequency of sampling that would be typical of implementation, as opposed to the frequent sampling required to quantify bacterial growth and distribution under different conditions. Also, this project included two separate drilling mobilizations in order to properly construct both treatment cells; this would not be required for a typical implementation.

The number of injection wells required was scaled up based on the demonstration. For the active approach, this was based on approximately one-half to two-thirds of the treatment cell being impacted during the demonstration using two extraction and two injection wells. For the theoretical site, this led to 10 injection and 10 extraction wells for the active approach, and 19 injection wells for the passive approach. The same lactate injection frequency as in the demonstration was assumed (weekly for active, and monthly for passive). Monitoring would be

conducted quarterly rather than the monthly demonstration monitoring. Also, CSIA would not be performed, and qPCR for *DHC* would only be performed during the first year of operations.

Tables 5 and 6 present the projected implementation costs for bioaugmentation using the active and passive approach, respectively. Most of the costs are similar because they are common to both active and passive approaches. However, for a theoretical site of this size, the construction and O&M costs for the active approach are approximately three times higher than for the passive approach. The result is an estimated cost for the active approach of \$2.5 million, compared to \$1.5 million for the passive approach. The primary drivers for this cost increase are the significantly higher amount of lactate required and the higher costs for maintenance and oversight of recirculation systems. The magnitude of the cost differences for O&M activities increases as the size of the area treated increases. As alluded to in Section 7, the benefits of implementing an active approach do not appear to be justified by the increased costs, at least for a site like NAVWPNSTA Seal Beach. Bacterial distribution was not significantly faster, and dechlorination performance was similar to the passive approach.

Table 5. Projected implementation costs for bioaugmentation using active recirculation.

Cost Element	Subcategory	Detail	Costs
Start-up costs	Treatability/DNA sequencing study	Procurement – 80 hr	\$6000
		Subcontractors (lab services)	\$20,000
	Work plan	Project manager – 220 hr	\$27,500
		Technical reviewer – 40 hr	\$8000
		Project engineer – 340 hr	\$34,000
		Drafting/clerical – 60 hr	\$4500
		Subtotal	\$100,000
	Well installation/development	Project geologist – 500 hr	\$50,000
		Subcontractor	\$112,000
		Materials/ODCs	\$20,000
	Tracer testing/hydraulic characterization	Project manager – 20 hr	\$2500
		Project engineer – 20 hr	\$2000
		Project geologist – 80 hr	\$8000
		Materials/ODCs	\$4200
	Screening level groundwater modeling	Project hydrogeologist – 24 hr	\$3000
		Subtotal	\$201,700
Active approach construction/O&M	Oversight/supervision	Project manager – 800 hr	\$100,000
	Lactate injection system purchase/construction	Subcontractor	\$160,000
	Lactate injection (1x every week)	Project engineer – 10 hr/event, 260 events	\$260,000
		Lactate – 250 gal per event, 260 events	\$780,000
	Bioaugmentation	Project engineer – 80 hr	\$8000
		Bacterial culture	\$60,000
	System troubleshooting/maintenance (1 major and 3 minor events during demo)	Project engineer – 320 hr	\$32,000
		Technician – 320 hr	\$19,200
		Materials/ODCs	\$40,000
	Sampling (21 total events)	Project engineer – 630 hr	\$63,000
		Project geologist – 630 hr	\$63,000
		Analytical (all analytes, excluding CSIA and qPCR only for Year 1)	\$135,000
		Materials/ODCs (\$1500 per event)	\$31,500
		Subtotal	\$1,751,700

Table 5. Projected implementation costs for bioaugmentation using active recirculation (continued).

Cost Element	Subcategory	Detail	Costs
Performance assessment, reporting, and project management	Includes final project reports, tech transfer, and data management/interpretation	Project manager – 1200 hr	\$150,000
		Technical reviewer – 400 hr	\$80,000
		Project engineer – 1200 hr	\$120,000
		Drafting/clerical – 400 hr	\$30,000
		Travel/ODCs	\$40,000
		Subtotal	\$420,000
Demobilization	Site cleanup and restoration		\$20,000
Waste disposal			NA
Long-term monitoring			NA
		Total	\$2,493,400

Table 6. Projected implementation costs for bioaugmentation using passive approach.

Cost Element	Sub-Category	Detail	Costs
Start-up costs	Treatability/DNA sequencing study	Procurement – 80 hr	\$6000
		Subcontractors (lab services)	\$20,000
	Work plan	Project manager – 220 hr	\$27,500
		Technical reviewer – 40 hr	\$8000
		Project engineer – 40 hr	\$34,000
		Drafting/clerical – 60 hr	\$4500
		Subtotal	\$100,000
General construction costs	Well installation/development	Project geologist – 500 hr	\$50,000
		Subcontractor	\$112,000
		Materials/ODCs	\$20,000
	Tracer testing/hydraulic characterization	Project manager – 20 hr	\$2500
		Project engineer – 20 hr	\$2000
		Project geologist – 80 hr	\$8000
		Materials/ODCs	\$4200
	Screening level groundwater modeling	Project hydrogeologist – 24 hr	\$3000
		Subtotal	\$201,700
Passive approach construction/O&M	Oversight/supervision	Project manager – 400 hr	\$50,000
	Lactate injection system purchase/construction	Subcontractor	\$62,000
	Lactate injection (1x every week)	Project engineer – 20 hr/event, 48 events	\$96,000
		Lactate – 317 gal per event, 48 events	\$182,400
	Bioaugmentation	Project engineer – 80 hr	\$8000
		Bacterial culture	\$60,000
	System troubleshooting/maintenance (1 major and 3 minor events during demo)	Project engineer – 40 hr	\$4000
		Technician – 40 hr	\$2400
		Materials/ODCs	\$4000
	Sampling (21 total events)	Project engineer – 630 hr	\$63,000
		Project geologist – 630 hr	\$63,000
		Analytical (all analytes, excluding CSIA and qPCR only for Year 1)	\$135,000
		Materials/ODCs (\$1500 per event)	\$31,500
		Subtotal	\$761,300

Table 6. Projected implementation costs for bioaugmentation using passive approach (continued).

Cost Element	Subcategory	Detail	Costs
Performance assessment, reporting, and project management	Includes final project reports, tech transfer, and data management/interpretation	Project manager – 1200 hr	\$150,000
		Technical reviewer – 400 hr	\$80,000
		Project engineer – 1200 hr	\$120,000
		Drafting/clerical – 400 hr	\$30,000
		Travel/ODCs	\$40,000
	Subtotal		\$420,000
Demobilization	Site cleanup and restoration		\$20,000
Waste disposal			NA
Long-term monitoring			NA
		Total	\$1,503,000

It should be noted that some sites might have conditions that would lead to more significant benefits for recirculation systems. For sites with very high groundwater flow velocities, recirculation might be needed to manage residence within the treatment zone to avoid chlorinated degradation products migrating offsite. Such a site would also allow electron donor to be distributed over a much larger distance prior to being degraded than was possible at Seal Beach, which would increase the benefit.

On the other hand, sites with very low groundwater velocities might make a passive system impractical because very little distribution can be achieved without enhancing the hydraulic gradient. What this demonstration indicates is that for sites that are closer to the average in terms of groundwater velocity, passive bioaugmentation systems are likely to be more cost-effective than active systems.

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9.0 IMPLEMENTATION ISSUES

This section discusses implementation issues for bioaugmentation. In general, the issues are similar when using either the passive or active approach. However, additional issues related to permitting may be encountered when applying the technology using the active recirculation approach.

The primary regulation or set of regulations that are applicable to bioaugmentation technology are related to underground injection control. Permits may be required for both electron donors and for bioaugmentation cultures. Specifically in California, Waste Discharge Requirement (WDR) permits are required. General WDR permit No. R4-2007-0019 covers groundwater remediation at petroleum hydrocarbon fuel, VOC, and/or hexavalent chromium impacted sites. Any amendment listed in this permit can be used at a site without a separate permitting process. In cases where a general WDR permit does not cover the amendments or cultures required for a site, a site-specific WDR permit may be needed. It should be noted that permits are not required for remediation at Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites such as NAVWPNSTA Site 70; however, the substantive requirements of the permits need to be met.

Bioaugmentation at sites that use recirculation also need to address the issue of how extracted water is handled. Some states may have regulations that state extracted water needs to be treated prior to reinjection. However, Resource Conservation and Recovery Act (RCRA) regulations (specifically 3020[b]) specifically allow for both injection of treatment agents and reinjection of extracted water amended with bioremediation treatment agents if certain conditions are met: “Specifically, the groundwater must be treated prior to reinjection; the treatment must be intended to substantially reduce hazardous constituents in the ground water—either before or after reinjection; the cleanup must be protective of human health and the environment; and the injection must be part of a response action under CERCLA, Section 104 or 106, or a RCRA corrective action intended to clean up the contamination.”

While bioaugmentation is an innovative technology that has not been extensively documented at full scale, in situ bioremediation has been implemented at many Department of Defense (DoD) sites across the country. In general, in situ bioremediation is well received by regulators and the public for many reasons, including:

- Low Risks – Since most or all of the contaminant treatment occurs in the soil or groundwater, risks to human health and the environment during implementation are low compared to ex situ technologies.
- Low secondary waste generation – Contaminant treatment occurs in situ, with little offsite disposal of residuals required.
- Minimal impacts during operations – Compared to ex situ technologies, little infrastructure is required to implement and operate the bioremediation systems, resulting in minimal disruption to businesses and residences.

- Overall risk reduction – In situ bioremediation has been shown to be reliable in significantly decreasing contaminant concentrations in relatively short time frames, resulting in reductions of risk to human health and the environment.

While the merits of bioremediation have resulted in widespread acceptance of the technology, full-scale bioaugmentation does present issues that are not encountered for bioremediation alone. These issues can be categorized as either concerns about the technology itself or decision-making factors related to implementation of the technology.

The primary concerns about full-scale bioaugmentation are related to the introduction of exogenous bacteria to a site's groundwater. Stakeholders may object to the introduction of non-native bacteria to an aquifer. For the current demonstration project, this concern was addressed by citing the precedence for performing bioaugmentation at other sites, most notably at NAVWPNSTA Seal Beach Site 40, as well as the fact that bioaugmentation is the CERCLA selected remedy for Site 70. Another concern related to the introduction of bacteria may be simply the ability to distribute them over a sufficient area to achieve full-scale treatment; this was the purpose of this demonstration project.

No significant procurement issues exist for bioaugmentation. This technology uses readily available techniques for well installation and standard components for performing substrate injections. Projects that use a recirculation approach require more equipment and aboveground infrastructure, but it is all standard and readily available from industrial supply companies. Amendments are widely available from bioremediation vendors across the country, and several bioaugmentation cultures are available from multiple suppliers. Bioaugmentation technology does require somewhat specialized expertise to properly interpret data and make operational changes in order to optimize performance.

10.0 REFERENCES

- Bechtel Environmental, Inc. 1999. Final Technical Memorandum 4, Groundwater Pumping Test Report, IR Site 70, Naval Weapons Station Seal Beach, CA.
- Bechtel Environmental, Inc. 2005. "Draft Fifth Annual Groundwater Monitoring Report IR Sites 40 and 70, Naval Weapons Station Seal Beach, Seal Beach, CA," CTO-0002/0842, November.
- CDM Smith (CDM). 2007. Demonstration Plan for a Low-Cost, Passive Approach for Bacterial Growth and Distribution for Large-Scale Implementation of Bioaugmentation. ESTCP Project ER-0513.
- Cupples, A.M., A.M. Spormann, and P.L. McCarty. 2003. "Growth of a *Dehalococcoides*-like microorganism on vinyl chloride and *cis*-dichloroethene as electron acceptors as determined by competitive PCR." *Appl. Environ. Microbiol.* 69:953-959.
- Drzyzga, O., J.C. Gottschalk. 2002. *Appl. Environ. Microbiol.* 68: 642-649.
- Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, Jr., and C.L. Bartlett. 2000. "Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation." *Environmental Science and Technology* 34(11): 2254-2260.
- Environmental Security Technology Certification Program (ESTCP). 2008. In Situ Bioremediation of Chlorinated Solvents with Enhanced Mass Transfer. Project ER-200218 Final Report.
- ESTCP. 2010. A Low-Cost, Passive Approach for Bacterial Growth and Distribution for Large-Scale Implementation of Bioaugmentation. Project ER-200513 Final Report.
- Freedman, D.L., and J.M. Gossett. 1989. *Appl. Environ. Microbiol.* 55, 2144-2151.
- He, J., K.M. Ritalahti, M.R. Aiello, and F.E. Löffler. 2003. "Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species." *Appl. Environ. Microbiol.* 69:996-1003.
- Hendrickson, E.R., J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnstock, D.E. Ellis, and R.C. Ebersole. 2002. "Molecular Analysis of *Dehalococcoides* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe." *Applied and Environmental Microbiology*, 68: 485-495.
- Holliger, C.W., G. Wohlfarth, and G. Diekert. 1999. Reductive dechlorination in the energy metabolism of anaerobic bacteria. *FEMS Microbio. Rev.* 22(5): 383-397.
- Interstate Technology & Regulatory Council (ITRC). 2005. Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones. BioDNAPL-1, Washington, DC. Interstate Technology & Regulatory Council, DNAPL team, available online at www.itrcweb.org.

Lendvay, J.M., F.E. Loeffler, M. Dollhopf, M.R. Aiello, G. Daniels, B.Z. Fathepure, M. Gebhard, R. Heine, R. Helton, J. Shi, R. Krajmalnik-Brown, C.L. Major, M.J. Barcelona, E. Petrovskis, R. Hickey, J.M. Tiedje, and P. Adriaens. 2003. Environmental Science and Technology, 37:1422-1431.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M. G. Starr, J.A. Payne, and L. Buanamici. 2002. Successful Bioaugmentation to Achieve Complete Dechlorination of Chlorinated Ethenes and Validation through Molecular Monitoring. Environmental Science and Technology, 36(23):5106-5116.

Maymó-Gatell, X., and S.H. Zinder. 2001. Environ. Sci. Technol. 35(3): 516-521.

Maymó-Gatell, X., T. Anguish, and S.H. Zinder. 1999. "Reductive Dechlorination of Chlorinated Ethenes and 1,2-Dichloroethane by 'Dehalococcoides ethenogenes' 195." Applied and Environmental Microbiology. 65:3108-3113.

Maymó-Gatell, X., Y.-T. Chien, J.M. Gossett, and S.H. Zinder. 1997. "Isolation of a Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene." Science. 276:1568-1571.

Sorenson, K.S. 2003. "Aqueous or Slow Release?—Considerations for Substrate Selection." Proceedings of the 2003 AFCEE Technology Transfer Workshop, San Antonio, TX, February.

APPENDIX A

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